Association of circulating osteocalcin with cardiovascular disease and intermediate cardiovascular phenotypes: systematic review and meta-analysis

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Abstract

Background: Circulating osteocalcin (OC), a marker which is central in bone mineralization, may be involved in the atherosclerotic process and influence the risk of developing cardiovascular disease (CVD).

Aims: We conducted a systematic review and meta-analysis of published observational evidence, to assess and quantify the associations of circulating OC (total, undercarboxylated, and carboxylated OC) with cardiovascular outcomes (clinical CVD endpoints and intermediate cardiovascular phenotypes).

Methods: Relevant studies were identified in a literature search of MEDLINE, EMBASE, and reference lists of relevant studies to March 2019. Mean differences and risk ratios with 95% CIs were aggregated using random-effects models.

Results: Thirty-three observational studies (prospective and retrospective cohort, case-control, and cross-sectional) with data on 21,021 unique participants were eligible. The pooled risk ratio in a comparison of extreme fourths of total OC levels was 0.98 (95% CI 0.89, 1.08) for composite CVD. Circulating total OC levels were significantly lower in patients with cardiovascular conditions compared with those without these conditions -2.58 ng/ml (95% CI -3.85, -1.32; $p<0.001$). Prospective and cross-sectional data showed significant inverse associations between total OC and traits such as aortic or coronary calcification, coronary atherosclerosis or calcification, carotid intima-media thickness, and plaque score. There was limited data on carboxylated and undercarboxylated OC, with no evidence of associations.

Conclusion: Observational evidence generally supports inverse associations of circulating total OC with risk of atherosclerotic outcomes and CVD endpoints; however, the data were mostly based on cross-sectional evaluations. Large-scale prospective data are needed.

Keywords: osteocalcin; cardiovascular disease; coronary heart disease; atherosclerosis; carotid intima-media thickness; meta-analysis

Introduction

Cardiovascular disease (CVD) is still the leading cause of global mortality.(1) By 2030, an estimated 23.6 million people will die from CVD.(2) Major risk factors for CVD include a history of diabetes, high blood pressure, raised blood lipids, as well as smoking status.(3) Though these established risk factors explain a large proportion of the risk of CVD, its pathogenesis is still not fully established as it appears other additional factors may be involved. Osteocalcin (OC), a bone matrix protein mainly expressed by osteoblasts(4) and used as a biochemical indicator of bone formation,(5) has been proposed as a potential biomarker of CVD risk. Osteocalcin is secreted by osteoblasts in a fully carboxylated form, which is then decarboxylated to a more active biological form (4, 6, 7). Circulating blood levels of total OC comprises both carboxylated and undercarboxylated OC. Serum OC has been investigated as a hormone which plays a role in regulating glucose metabolism and fat mass; it promotes insulin sensitivity and secretion by pancreatic β-cells, as well as increases proliferation of the β-cells and stimulates energy metabolism.(6) Reduced circulating levels of OC have been consistently linked with high blood glucose levels, insulin resistance, and type 2 diabetes.(6, 8-10). Indeed, it has been discussed that OC may hold potential for the prevention, delay and treatment of obesity and metabolic disorders such as diabetes.(8) Emerging evidence also suggests circulating OC may be involved in CVD development, but the data are sparse and conflicting. A number of studies have shown reduced levels of circulating OC to be associated with adverse cardiovascular outcomes such as arterial calcification, carotid atherosclerosis, increased carotid intima-media thickness (CIMT), and CVD;(11-13) whereas others have not demonstrated any relationships.(14, 15). In this context, we performed a systematic review and meta-analysis of all available published observational evidence to clarify and quantify the extent of potential associations of circulating OC (total, uncarboxylated, and carboxylated OC) with (i) clinical CVD outcomes as well as all-cause mortality; and (ii) intermediate cardiovascular traits. We also sought to identify gaps in the existing evidence.

Methods

Data sources and search strategy

This review was conducted using a predefined protocol and in accordance with PRISMA and MOOSE guidelines (16, 17) (**Appendix 1-2**). We searched MEDLINE and EMBASE up to 22 March 2019. The computer-based searches combined terms related to the exposure (e.g., "osteocalcin") and outcomes (e.g., "cardiovascular disease", "coronary heart disease", "atherosclerosis", "carotid intima-media thickness", "mortality") in humans, without any language restriction. Reference lists of selected studies and relevant reviews on the topic were manually scanned for additional publications missed by the original search. Full details on the search strategy are presented in **Appendix 3**.

Eligibility criteria

We systematically searched for observational cohort, case-control, or cross-sectional population-based studies that had reported on associations of circulating levels of OC (total OC, undercarboxylated OC, and carboxylated OC) with (i) CVD-related outcomes [composite CVD, coronary heart disease (CHD), stroke, congestive heart failure (CHF), or all-cause mortality]; and (ii) intermediate cardiovascular traits [carotid atherosclerosis, aortic atherosclerosis, coronary artery calcification (CAC), carotid intima-media thickness (CIMT), abdominal aortic calcification (AAC)].

Data extraction and quality assessment

The titles and abstracts of all articles identified by the broad literature search were assessed independently by two reviewers (SKK and SS). Studies that did not meet inclusion criteria were discarded. Full text of selected articles were retrieved and assessed to determine if they met the inclusion criteria. Those studies which met the inclusion criteria were included in the review and data was extracted independently by two reviewers (SKK and SS) using a standard data extraction form. The quality of the studies was assessed independently by both reviewers

When available, data were extracted on: publication date; study design; geographical location; population source; year of baseline survey; sample population; mean/median age at baseline; duration of follow-up (for cohort studies); type of OC; mean/median levels of OC; type of outcome; and reported risk estimates. In the case of multiple publications involving the same study, data were extracted from the most up-to-date study or study with the most comprehensive information was abstracted. We also corresponded with study investigators to provide missing information where relevant. Any discrepancies regarding eligibility of an article were discussed, and consensus reached with a third reviewer (K.K.). For cohort and case-control studies, study quality was assessed based on the nine-star Newcastle–Ottawa Scale (NOS) (18) using three pre-defined domains namely: selection of participants (population representativeness), comparability (adjustment for confounders), and ascertainment of outcomes of interest. The NOS assigns a maximum of four points for selection, two points for comparability, and three points for outcome. Nine points on the NOS reflects the highest study quality. For cross-sectional studies, quality was evaluated using the NOS modified for cross-sectional studies (**Appendix 4**(10)). A score of 8 reflected the highest study quality.

Statistical analysis

Mean differences for continuous outcomes and risk ratios for categorical outcomes were used as summary measures across studies. A narrative synthesis was performed for studies that could not be pooled. For data reported as medians, standard errors, ranges, and 95% confidence intervals (CIs), means and standard deviations were estimated using methods as described by Hozo and colleagues (19). To enable a consistent approach to the meta-analysis, enhance comparability and interpretation of the findings, units of measurements were converted where appropriate and reported study-specific risk ratios (per-unit or standard deviation change, quintiles, or other groupings) were also transformed to involve comparisons between the top quartile and bottom quartile of each study population's baseline distribution of OC levels, using standard statistical methods.(20, 21) described previously.(22-24) To account for the effect of between-study heterogeneity anticipated, we used the

inverse variance weighted method to combine summary measures using random-effects models.(25) We evaluated for publication bias using funnel plots and Egger's regression symmetry tests (26). All tests were two-tailed and p-values of 0.05 or less were considered significant. STATA release 14 (Stata Corp, College Station, Texas, USA) was used for all statistical analyses.

Results

Study identification and selection

165 potentially relevant citations were identified. 40 articles were selected for full text evaluation after an initial screen based on titles and abstracts. After detailed assessments, 8 articles were excluded because (i) the outcomes were not relevant to review (n=5); (ii) the exposure was not relevant (n=2); and (ii) one article used the same population sample as another study included in the review. The remaining 32 articles based on 33 unique observational studies met all inclusion criteria and were included in the review (**Figure 1; Appendix 5**).

Study characteristics and study quality

Table 1 shows a summary of the key characteristics of the included. 21,021 unique participants were included in this review. However, not all studies provided relevant data that could be included in the quantitative synthesis. The majority of studies (n=19) were conducted in Asia (China, Japan, and South Korea); with 10 in Europe (Austria, France, Germany, Italy, The Netherlands, and Spain); 2 in the Pacific (Australia); and 2 in North America (USA). The mean/median baseline age of participants ranged from 49 to 77 years. The majority of studies (n=20) were cross-sectional in design; 9 prospective cohorts; 2 case-controls; 1 retrospective cohort and 1 prospective case-control. The average follow-up for cohort studies ranged from 0.5 to 10.0 years. For studies that provided relevant data on their recruitment processes, majority of studies (n=22) reported recruiting patients from healthcare settings, with 7 studies reporting recruitment from a population register. There was considerable variability in study populations which included healthy and community-dwelling

participants, post-menopausal women, and patients with pre-existing conditions such as type 2 diabetes, chronic kidney disease, essential hypertension and those at high cardiovascular risk. Among cohort, case-control, and cross-sectional studies, quality score ranged from 3 to 8.

Association of circulating osteocalcin with cardiovascular outcomes

Cohort analysis The pooled risk ratio of 5 cohort studies (3481 participants and 686 events) for composite CVD risk comparing individuals in the top versus bottom fourths of circulating total OC levels, adjusted for several established cardiovascular risk factors was 0.98 (95% CI 0.89, 1.08; p=0.669) (**Figure 2**). The prospective corresponding risk ratios for CHD and stroke based on results of a single study were 1.06 (95% CI 0.38, 2.93) and 0.65 (95% CI 0.29, 1.46) respectively.

In pooled analysis of 2 prospective studies (1,760 participants and 378 events), the multivariate-adjusted risk ratio for all-cause mortality was 0.33 (95% CI 0.03, 3.51; p=0.355) comparing individuals in the top versus bottom fourths of circulating total OC levels. Four studies could not be included in the meta-analysis because of differences in the exposure categories. In analysis adjusted for several conventional risk factors, Yeap and colleagues reported a U-shaped relationship of circulating total OC with CVD mortality and all-cause mortality in men – the risk being increased at both ends of the distribution of OC levels.(27). In a follow-up of the same study that evaluated CHD and stroke outcomes, total OC was not associated with MI or stroke events.(15) In the Ludwigshafen Risk and Cardiovascular Health (LURIC) prospective cohort study,(28) findings reported in men showed a U-shaped association of total OC with fatal CVD and all-cause mortality - with increased risk at both ends of the distribution of OC levels. In a prospective case-control study of 102 MI patients and 200 control subjects, total OC was demonstrated to be associated with premature MI at one-year follow-up.(29)

In the single prospective cohort study that evaluated the associations between undercarboxylated OC and cardiovascular endpoints, there was no evidence of an association of undercarboxylated OC with CHD or stroke.(15)

Cross-sectional analysis Comparing individuals in the top versus bottom fourths of circulating total OC levels, the multivariate-adjusted risk ratio for CHD in pooled analysis of 3 cross-sectional studies (766 participants and 383 events) was 1.20 (95% CI 0.32, 4.50; p=0.789).

Association of serum total osteocalcin with intermediate cardiovascular traits

Cohort analysis The pooled risk ratio for aortic calcification in pooled analysis of 2 prospective cohort studies was 0.87 (95% CI 0.76, 0.99; p=0.030) comparing individuals in the top versus bottom fourths of circulating total OC levels. In a prospective cohort study that monitored changes in total OC and plaque score in Japanese patients with type 2 diabetes, changes in circulating total OC was inversely associated with changes in plaque score from baseline in analysis adjusted for atherosclerotic risk factors (β=-0.30; p=0.047).(30)

Cross-sectional analysis In pooled analysis of 8 studies (3356 participants), the multivariate-adjusted risk ratio for vascular atherosclerosis or calcification when comparing individuals in the top versus bottom fourths of circulating total OC levels was 0.67 (95% CI 0.37, 1.22; p=0.187) (**Figure 3**). The risk ratio for vascular atherosclerosis or calcification when comparing individuals in the top versus bottom fourths of circulating undercarboxylated OC levels in pooled analysis of 4 studies (762 participants) was 0.76 (95% CI 0.13, 4.63; p=0.767) (**Figure 3**).

Five studies could not be included in the meta-analysis. Kanazawa and colleagues in their assessment of the associations of serum total OC with atherosclerosis parameters in patients with type 2 diabetes, total OC was significantly and inversely correlated with CIMT in men (r=-0.181; p=0.023), but not in women (r=0.022; p=0.803).(31) In a sample of 817 men and postmenopausal women with type 2 diabetes, serum total OC was independently and inversely associated with CIMT (β=-0.181; r=-0.187; p<0.001).(12) Yang and colleagues also demonstrated a significant and inverse association between serum total OC and CIMT (β=-0.117; r=-0.107; p<0.01)(13). Kim and colleagues also reported total OC to be significantly and inversely correlated with aortic calcification as

measured by the aortic calcium score (r=-0.238; p<0.001).(11) An independent and inverse correlation (β=-0.497; p=0.003) was also demonstrated between serum total OC and coronary atherosclerosis index in the study by Bao and colleagues.(32)

Circulating osteocalcin levels in patients with cardiovascular outcomes and traits compared with controls

The pooled random-effects mean difference across 9 studies showed significantly lower circulating levels of total OC -2.58 ng/ml (95% CI -3.85, -1.32; p<0.001) in patients with cardiovascular conditions compared to subjects without cardiovascular conditions (**Figure 4**). In pooled analysis of 4 studies, there was no significant difference in circulating undercarboxylated OC levels comparing subjects with and without cardiovascular conditions. In a case-control study, circulating carboxylated OC level was significantly lower comparing subjects with and without CAD -0.50 ng/ml (95% CI -0.71, -0.29; p<0.001).

Discussion

Summary of findings

Using a systematic and meta-analytical approach, we have summarized all available observational studies that have assessed the associations of circulating levels of OC (total, undercarboxylated, and carboxylated OC) with clinical CVD endpoints and intermediate CVD traits, in an attempt to address the uncertainties in the evidence. In the analysis of hard CVD outcomes, pooled analysis of prospective data showed no evidence of statistically significant associations of total OC with composite CVD, CHD, or stroke endpoints. However, two prospective studies conducted in men demonstrated a U-shaped association of total OC with fatal CVD and all-cause mortality.(27, 28) Both low and high levels of total OC were associated with an increased risk of these outcomes. In the evaluation of intermediate cardiovascular traits, prospective data showed significant inverse associations between total OC and traits such as aortic calcification and plaque score. Though the

evidence from cross-sectional studies was inconsistent, majority of studies reported inverse, independent, and significant correlations between total OC and outcomes such as CIMT, aortic calcification, and coronary calcification or atherosclerosis. Circulating total OC was significantly lower in patients with cardiovascular conditions compared with those without these conditions. The data was limited for undercarboxylated and carboxylated OC and generally no significant evidence of associations were demonstrated with the outcomes assessed.

Interpretation of findings

The current evidence does not conclusively support a role of circulating OC (particularly total OC) in the pathophysiology of adverse cardiovascular lesions as well as CVD risk. Though some of the findings show that reduced levels of circulating total OC are associated with greater pathological cardiovascular changes, there was evidence to suggest that increased levels of total OC might also be associated with increased risk of these adverse outcomes. Beyond its well-established pro-osteoblastic functions(33), OC has been demonstrated to have endocrine functions.(6) Consistent evidence shows that reduced circulating levels of OC is associated with increased risk of adverse metabolic outcomes such as type 2 diabetes and metabolic syndrome (MetS), which is via its role in influencing insulin secretion, glucose metabolism, insulin sensitivity, fat mass, beta cell proliferation, and energy expenditure.(6, 34, 35) Though the mechanistic evidence has mostly involved the use of animal models, recent epidemiological and genetic studies suggest the multiple aspects of the biology of OC are similar for both humans and rodents. (36, 37) However, the mechanistic evidence linking OC with atherosclerotic CVD is unclear compared with metabolic conditions such as type 2 diabetes; a number of pathways have been proposed. Conditions such as insulin resistance, metabolic syndrome, non-alcoholic fatty liver disease, and type 2 diabetes, which are closed linked to circulating OC, accelerate the progression of atherosclerotic lesions and are strongly linked to the development of atherosclerosis.(12) Osteocalcin may be involved in the calcification process at arterial and valvular sites, leading to reduced elasticity and compliance of the vasculature.(38, 39) Osteocalcin may also

play a direct role in the atherosclerotic process, as osteoclast-like cells have been identified in atherosclerotic lesions.(39) Whether the different forms of OC may play different roles in the pathophysiology of adverse cardiovascular outcomes is not clear, as the evidence has mostly been limited to circulating total OC. However, there is evidence from animal models suggesting that undercarboxylated OC, the more active form of OC, may be of more importance in regulating glucose and energy metabolism(6, 34, 40) and therefore could be implicated in the development of adverse metabolic outcomes. This is not well established as it is not even certain if undercarboxylated OC might be the active form in humans (36).

Implications of findings

The current findings provide further insight on the role of OC beyond its pro-osteoblastic properties as well as its role in glucose and energy metabolism. The clinical use of circulating OC assays has mainly been for monitoring and assessing the effectiveness of antiresorptive therapy in patients with bone conditions characterised by elevated OC levels such as osteoporosis and Paget's disease.(41) Though some of the findings were inconsistent which impeded meaningful interpretation, the overall results of the quantitative synthesis of the available data does not underscore a potentially protective role of increased circulating total OC levels on the risk of atherosclerotic lesions and CVD outcomes. The inconsistencies in the findings could be related to the small sample sizes employed by majority of studies, different populations used, different OC assay methods employed, and the fact that circulating OC levels are influenced by ethnicity, gender and menopausal status.(42) There is some suggestion from our results that there may be a more evident association in males. In addition to highlighting the lack of consistent evidence on the cardiovascular effects of OC, this review has also identified several gaps in the literature. Compared with the number of cross-sectional study designs (n=20), only 10 observational cohort studies were identified to have evaluated the associations and these were limited by small sample sizes and populations with pre-existing disease. Furthermore, the majority of studies used assays for total OC, and therefore the role of other forms of OC is far from clear. This

observation could be attributed to the fact that circulating total OC can be conveniently measured in large studies using automated immunoassays (43), while assays for undercarboxylated OC are more cumbersome, labour intensive, or less precise (44). Large-scale prospective studies conducted in general population settings are needed to address the existing research gaps. Thus far, the potential of strategies that raise concentrations of circulating total OC and their value in the prevention or treatment of vascular diseases is not convincing enough. This is a topic which deserves further investigation.

Strengths and limitations

The strengths and limitations of this systematic meta-analysis merit careful consideration. The notable strengths included the comprehensive search strategy which yielded several published studies on the topic. Overall, this review involved over 21,000 participants and evaluated a wide-range of atherosclerotic and CVD outcomes and their relationships with the different forms of circulating OC. We were able to transform reported risk estimates from majority of contributing studies to a consistent comparison (mean differences and top versus bottom fourths) to allow a consistent combination of estimates across studies, therefore obtaining a reliable estimate of the magnitude of the association and enhancing interpretation of the overall findings. We also conducted a detailed quality assessment of eligible studies. Limitations included the inability to fully examine the impact of adjustment for potential confounding factors, because the review was based on variably adjusted data reported in the published literature; heterogeneity could not be explored because of the limited number of studies available for pooling; and some outcomes and risk estimates were inconsistent between studies and therefore could not be pooled.

In conclusion, aggregate observational data do not generally support inverse and independent associations of circulating total OC with risk of atherosclerotic outcomes and CVD endpoints; however, the data were mostly based on cross-sectional evaluations. There are gaps in the existing

literature and large-scale prospective cohort studies are needed to explore the nature and potential magnitude of any association of circulating OC with cardiovascular outcomes.

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Conflicts of Interest/Disclosures

None

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Figure legends

Figure 1. Selection of studies included in the meta-analysis

Figure 2. Association of circulating total osteocalcin with cardiovascular outcomes and all-cause mortality

CI, confidence interval (bars); OC, osteocalcin

Figure 3. Associations of circulating total and undercarboxylated osteocalcin with intermediate cardiovascular traits

AAC, abdominal aortic calcification; CAC, coronary artery calcification; CI, confidence interval (bars); OC, osteocalcin

Figure 4. Mean differences in circulating osteocalcin levels comparing subjects with cardiovascular conditions and their respective controls

CAC, coronary artery calcification; CAD, coronary artery disease; CHD, coronary heart disease; CHF, congestive heart failure; CI, confidence interval (bars)

Figure 1

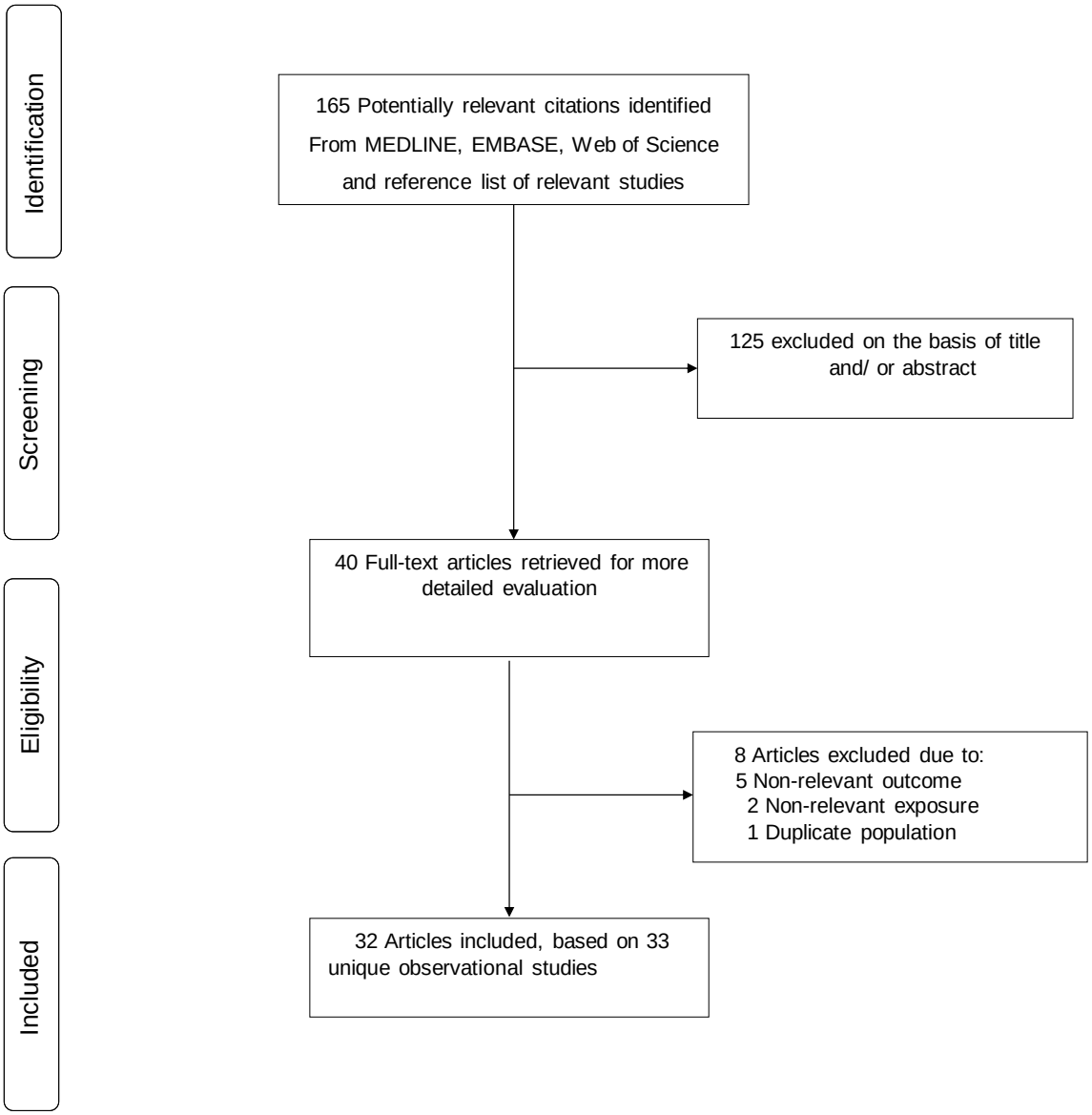
Identification
Screening
Eligibility
Included
165 Potentially relevant citations identified
From MEDLINE, EMBASE, Web of Science
and reference list of relevant studies
125 excluded on the basis of title
and/ or abstract
40 Full-text articles retrieved for more
detailed evaluation
8 Articles excluded due to:
5 Non-relevant outcome
2 Non-relevant exposure
1 Duplicate population
32 Articles included, based on 33
unique observational studies

Figure 2

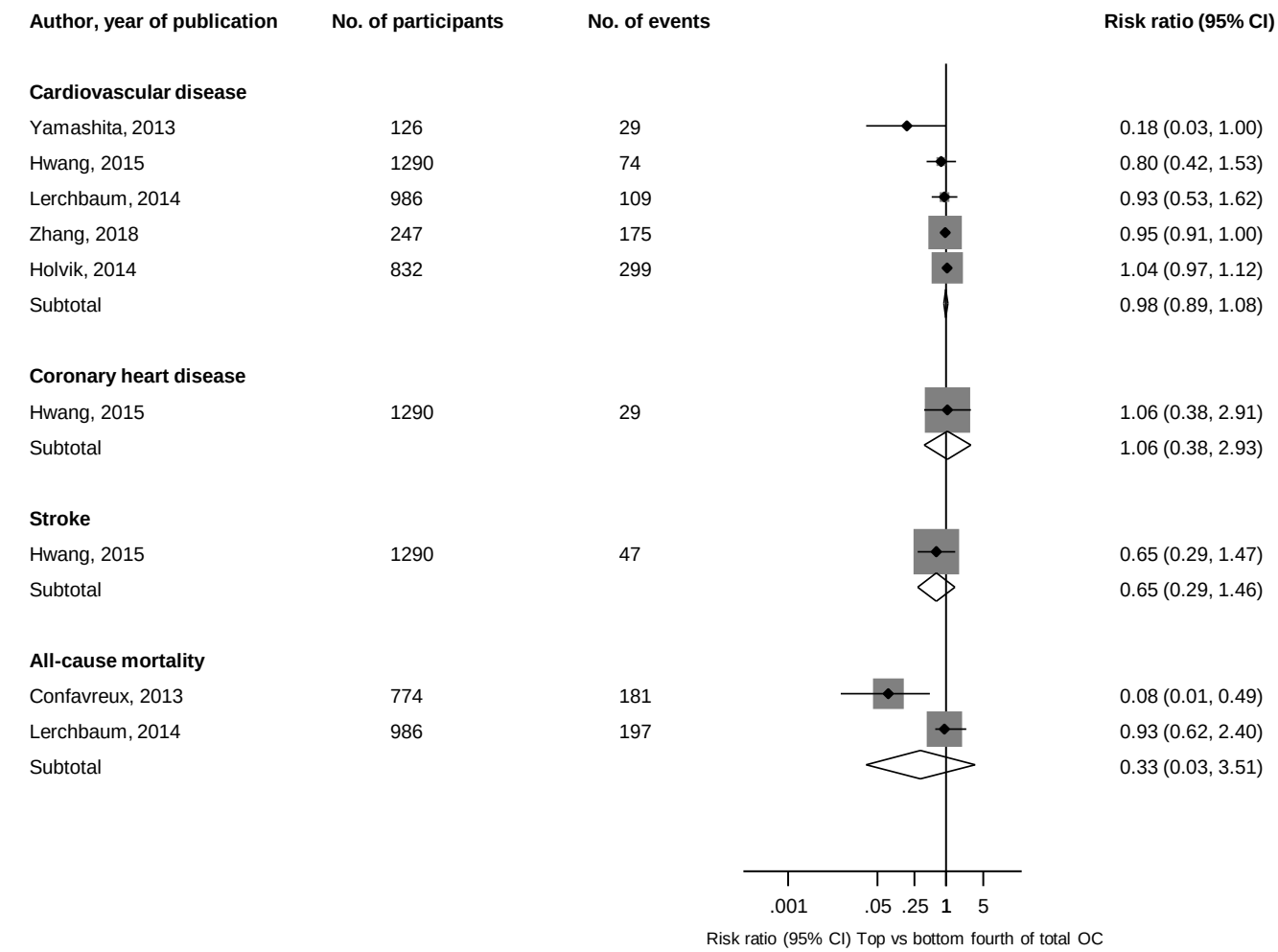
Author, year of publication
No. of participants
No. of events
Risk ratio (95% CI)
Cardiovascular disease
Yamashita, 2013 126 29 0.18 (0.03, 1.00)
Hwang, 2015 1290 74 0.80 (0.42, 1.53)
Lerchbaum, 2014 986 109 0.93 (0.53, 1.62)
Zhang, 2018 247 175 0.95 (0.91, 1.00)
Holvik, 2014 832 299 1.04 (0.97, 1.12)
Subtotal 0.98 (0.89, 1.08)
Coronary heart disease
Hwang, 2015 1290 29 1.06 (0.38, 2.91)
Subtotal 1.06 (0.38, 2.93)
Stroke
Hwang, 2015 1290 47 0.65 (0.29, 1.47)
Subtotal 0.65 (0.29, 1.46)
All-cause mortality
Confavreux, 2013 774 181 0.08 (0.01, 0.49)
Lerchbaum, 2014 986 197 0.93 (0.62, 2.40)
Subtotal 0.33 (0.03, 3.51)
.001
.05
.25
1
5
Risk ratio (95% CI) Top vs bottom fourth of total OC

Figure 3

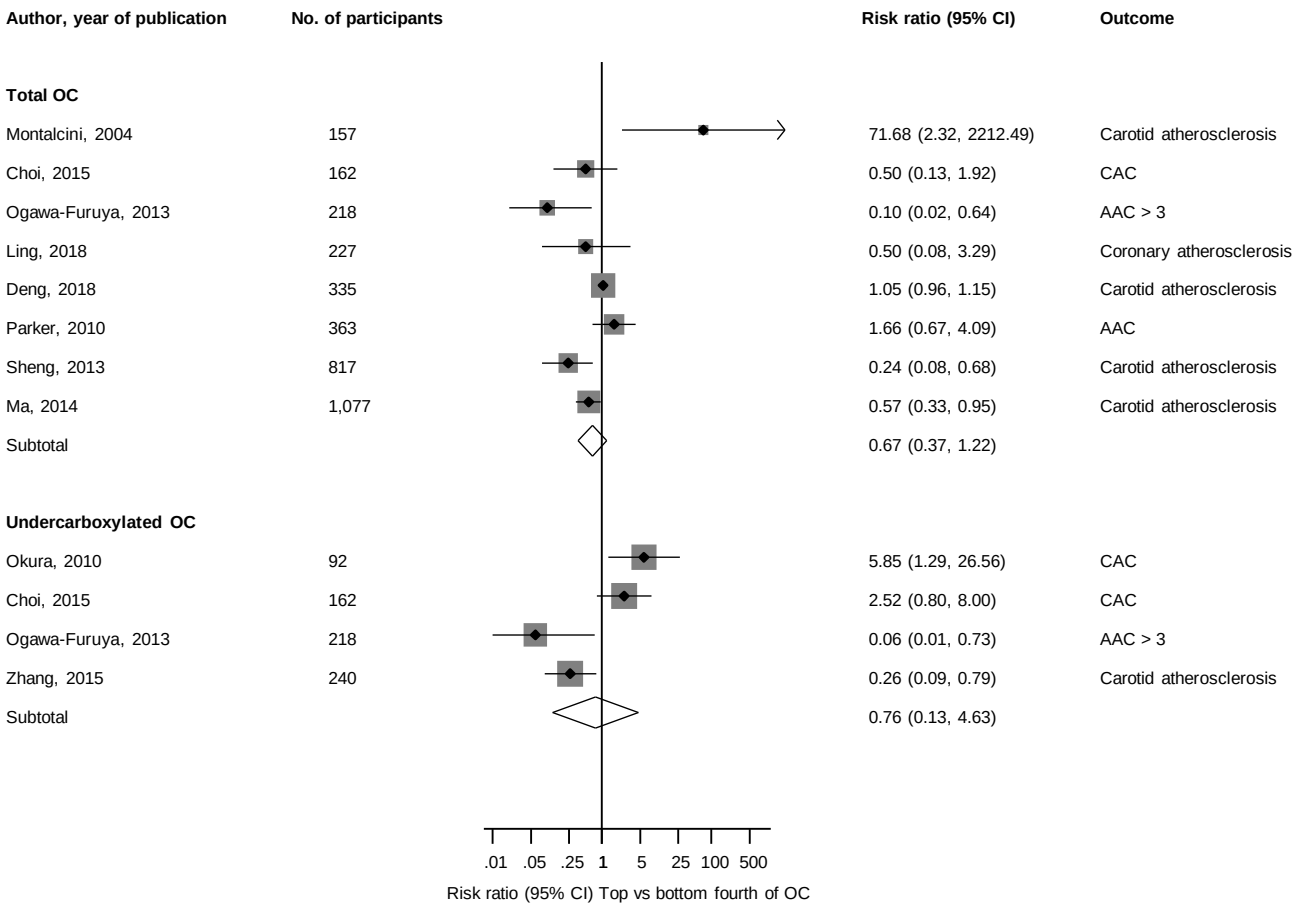
Author, year of publication
No. of participants
Risk ratio (95% CI)
Outcome
Total OC
Montalcini, 2004 157 71.68 (2.32, 2212.49) Carotid atherosclerosis
Choi, 2015 162 0.50 (0.13, 1.92) CAC
Ogawa-Furuya, 2013 218 0.10 (0.02, 0.64) AAC > 3
Ling, 2018 227 0.50 (0.08, 3.29) Coronary atherosclerosis
Deng, 2018 335 1.05 (0.96, 1.15) Carotid atherosclerosis
Parker, 2010 363 1.66 (0.67, 4.09) AAC
Sheng, 2013 817 0.24 (0.08, 0.68) Carotid atherosclerosis
Ma, 2014 1,077 0.57 (0.33, 0.95) Carotid atherosclerosis
Subtotal 0.67 (0.37, 1.22)
Undercarboxylated OC
Okura, 2010 92 5.85 (1.29, 26.56) CAC
Choi, 2015 162 2.52 (0.80, 8.00) CAC
Ogawa-Furuya, 2013 218 0.06 (0.01, 0.73) AAC > 3
Zhang, 2015 240 0.26 (0.09, 0.79) Carotid atherosclerosis
Subtotal 0.76 (0.13, 4.63)
.01 .05 .25 1 5 25 100 500
Risk ratio (95% CI) Top vs bottom fourth of OC

Figure 4

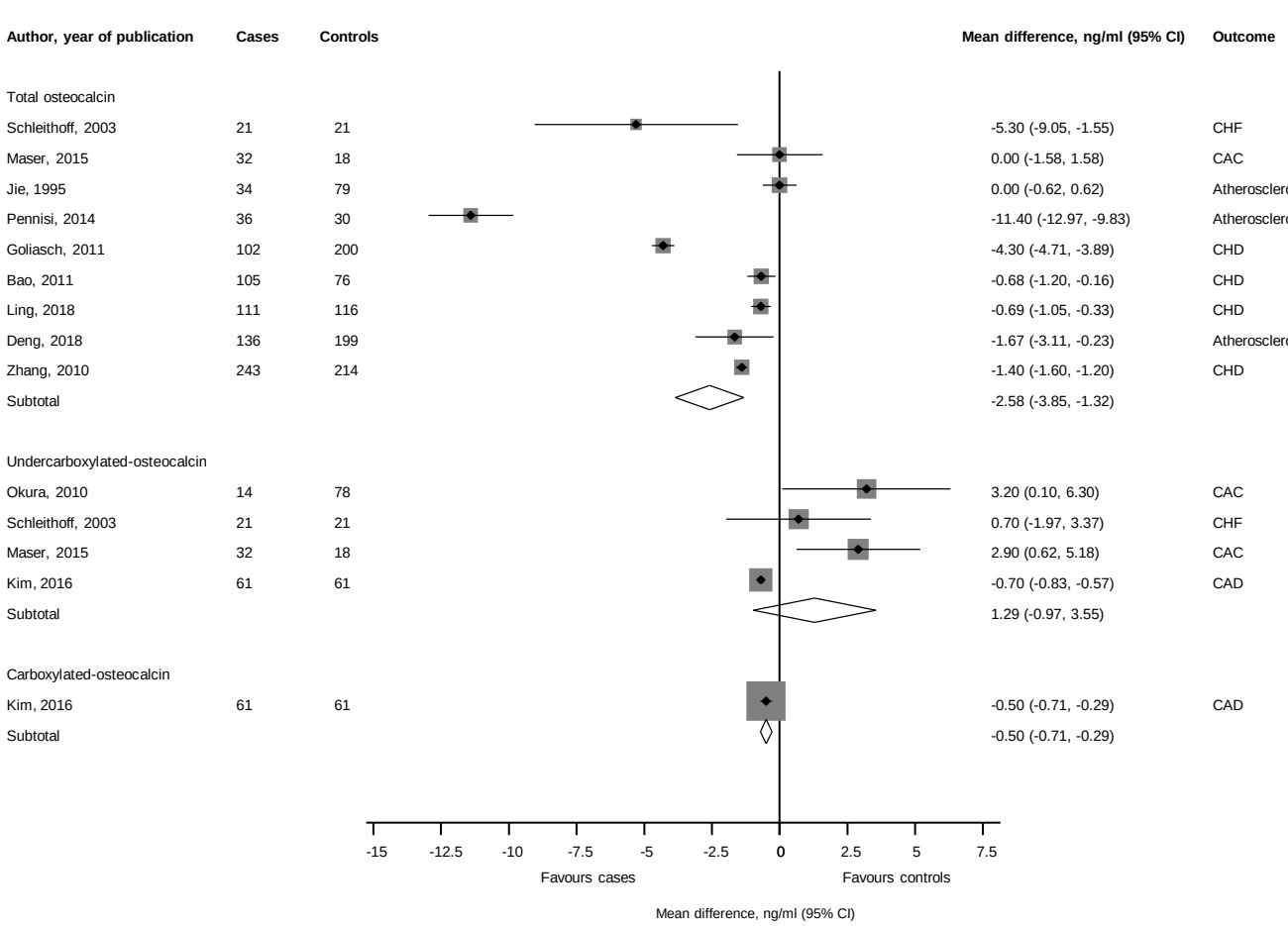
Author, year of publication
Cases
Controls
Mean difference, ng/ml (95% CI)
Outcome
Total osteocalcin
Schleithoff, 2003 21 21 -5.30 (-9.05, -1.55) CHF
Maser, 2015 32 18 0.00 (-1.58, 1.58) CAC
Jie, 1995 34 79 0.00 (-0.62, 0.62) Atherosclerosis
Pennisi, 2014 36 30 -11.40 (-12.97, -9.83) Atherosclerosis
Goliasch, 2011 102 200 -4.30 (-4.71, -3.89) CHD
Bao, 2011 105 76 -0.68 (-1.20, -0.16) CHD
Ling, 2018 111 116 -0.69 (-1.05, -0.33) CHD
Deng, 2018 136 199 -1.67 (-3.11, -0.23) Atherosclerosis
Zhang, 2010 243 214 -1.40 (-1.60, -1.20) CHD
Subtotal -2.58 (-3.85, -1.32)
Undercarboxylated-osteocalcin
Okura, 2010 14 78 3.20 (0.10, 6.30) CAC
Schleithoff, 2003 21 21 0.70 (-1.97, 3.37) CHF
Maser, 2015 32 18 2.90 (0.62, 5.18) CAC
Kim, 2016 61 61 -0.70 (-0.83, -0.57) CAD
Subtotal 1.29 (-0.97, 3.55)
Carboxylated-osteocalcin
Kim, 2016 61 61 -0.50 (-0.71, -0.29) CAD
Subtotal -0.50 (-0.71, -0.29)
-15
-12.5
-10
-7.5
-5
-2.5
0
2.5
5
7.5
Favours cases
Favours controls
Mean difference, ng/ml (95% CI)

Table 1. Characteristics of studies included in review

Lead Author, Publication Date	Name of study or source of participants	Location	Study design	Baseline population	Year of baseline survey	Baseline age range (years)	% male	Follow up (years)	Outcome (s)	Total participants	Covariates adjusted for	Study quality
Jie, 1995	Population-based	Netherlands	Cross-sectional	Post-menopausal	1990	66.6*	0.0	NA	Aortic atherosclerosis	113	NA	4
Schleithoff, 2003	Health setting	Germany	Case-control	CHF patients and controls	2000-2001	66.2-71.5*	100.0	NA	CHF	42	NA	4
Montalcini, 2004	Hospital clinic	Italy	Cross-sectional	Post-menopausal women	NR	45-75	0.0	NA	Carotid atherosclerosis	157	Age, hypertension, hyperlipidemia, type 2 diabetes, obesity, smoker	6
Pennisi, 2004	Hospital setting	Italy	Case-control	Atherosclerotic plaques and/or vascular calcification	2003	62.7*	NR	NA	Carotid/femoral atherosclerosis	66	NA	5
Kanazawa, 2009	Hospital	Japan	Cross-sectional	Type 2 diabetes	NR	50-83 50-84	100.0 0.0	NA	CIMT	179 149	Age, duration of diabetes, BMI, serum creatinine, systolic blood pressure, LDL-C, HDL-C, HbA1C, and Brinkmann index	5
Okura, 2010	NR	Japan	Cross-sectional	Essential hypertension	NR	61.0*	58.0	NA	CAC	92	NR	4
Parker, 2010	SOF	USA	Cross-sectional	Community-dwelling postmenopausal	1986-1988	≥ 65	0.0	NA	AAC	363	Age, body mass index, systolic and diastolic blood pressure, diabetes, hypertension, smoking history, eGFR, CRP, estrogen use, intact parathyroid hormone, bone-specific alkaline phosphatase, and 25-hydroxyvitamin D; hip and spine bone mineral density	6
Zhang, 2010	NR	China	Cross-sectional	Subjects referred for coronary angiography	2005-2007	39-85	64.9	NA	CHD	461	Age, gender, BMI, smoking, alcohol, family history of CHD, LDL-C, HDL-C, TG, type 2 diabetes	6
Bao, 2011	Hospital	China	Cross-sectional	Coronary angiography patients	2008-2009	64.9*	100.0	NA	Coronary artherosclerosis index	181	Age, BMI, fasting insulin, HbA1c, HOMA-IR	6
Goliasch, 2011	Hospital setting	Austria	Case-control	MI patients with controls	2004-2008	≤40	88.7	NA	NA	302	NA	8
Kanazawa, 2011	NR	Japan	Prospective cohort	Type 2 diabetes	NR	64.5*	56.0	0.5	Plaque score	50	Duration of diabetes, Brinkman Index	5
Kim, 2012	SBMS	South Korea	Cross-sectional	Subjects referred for QCT	2007-2010	64.7*	0.0	NA	Aortic atherosclerosis	769	NA	4

Lead Author, Publication Date	Name of study or source of participants	Location	Study design	Baseline population	Year of baseline survey	Baseline age range (years)	% male	Follow up (years)	Outcome (s)	Total participants	Covariates adjusted for	Study quality
Reyes-Garcia, 2012	Endocrinology Unit	Spain	Cross-sectional	Type 2 diabetes	2006-2007	57.8*	55.1	NA	CHD	78	Age, gender, BMI, hypt, smoking, DM duration, aortic calcifications, abnormal IMT, carotid plaques	6
Yeap, 2012	HIMS	Australia	Prospective cohort	Community-dwelling elderly men	2001-2004	77.0*	100.0	5.2	CVD, all-cause mortality	3,542	Age, WHR, conventional CVD risk factors	8
Confavreux, 2013	MINOS	France	Prospective cohort	Elderly men	1995-1996	51-85	100.0	10	AAC, all-cause mortality	774	Age, diabetes, hip to waist ratio, hypertension, smoking status, alcohol intake, serum phosphate, low HDL-cholesterol, AAC score, physical activity, and 25OHD	7
Lerchbaum, 2013	LURIC	Germany	Prospective cohort	Suspected CAD	1997-2000	58.72	100.0	7.7	CVD mortality, all-cause mortality	2,271	Age, BMI, smoking, WHR, CETP, hs-CRP, homocysteine, interleukin-6, HDL cholesterol, LDL cholesterol, triglycerides, prevalent metabolic syndrome	8
Ogawa-Furuya, 2013	Hospital	Japan	Cross-sectional	Type 2 diabetes	2005-2011	64.0*	54.1	NA	AAC	218	Age, BMI, creatinine, LDL-C, radial BMD, smoking, duration of DM, HbA1c, HOMA-IR, BAP, UNTx	6
Sheng, 2013	Shanghai downtown residential areas	China	Cross-sectional	Type 2 diabetes	NR	>50	46.8	NA	Carotid atherosclerosis, CIMT	817	Age, sex, smoking, alcohol, family history of diabetes, WC, HOMA-IR, BMI, WHR, TG, TC, HDL-C, LDL-C, CRP, treatments	6
Yamashita, 2013	Outpatient dialysis center	Japan	Prospective cohort	Maintenance haemodialysis patients	2005	20-85	61.1	5.0	CVD	126	Age, sex, time on dialysis, previous CVD, diabetes, serum Calcium x phosphate product, serum parathyroid hormone levels	7
Yang, 2013	SOS	China	Cross-sectional	Post-menopausal women	NR	41-78	0.0	NA	CIMT	1,319	Age, years since menopause, BMI, WC, SBP, DBP, HOMA-IR, TG, HDL-C, CRP, smoking, treatment, family history of CVD	7
Holvik, 2014	LASA	Netherlands	Prospective cohort	Elderly participants	1995-1996	65-88	48.8	4.1	CVD, ACS	832	Age, BMI, SBP, TG, TC, HDL-C, fructosamine	8
Lerchbaum, 2014	LURIC	Germany	Prospective cohort	Suspected CAD	1997-2000	58.72	0.0	7.7	CVD mortality, all-cause mortality	986	Age, BMI, menopause, prevalent CAD, HRT, vitamin K antagonist use	8
Ma, 2014	Shangai Changfeng study	China	Cross-sectional	Middle-aged and elderly	2009-2012	61.3*	100.0	NA	Carotid atherosclerosis	1,077	Age, FPG, PPG, BMI, WHR, smoking, SBP, DBP, TG, HDL-C, LDL-C, HOMA-IR, HOMA-B	7
Choi, 2015	Hospital	South Korea	Cross-sectional	Asymptomatic patients	2010-2012	53.5	70.4	NA	CAC	162	Age, body mass index, smoking (menopause), hypertension, diabetes, systolic blood pressure, HOMA2-IR, triglycerides, HDL cholesterol and lumbar BMD	6
Hwang, 2015	HPC	Korea	Prospective cohort	Middle-aged men	1997	40-78	100.0	8.7	Composite CVD, CHD, stroke	1,290	Age, BMI, current smoking, LDL-C, diabetes, hypertension, serum creatinine	7

Lead Author, Publication Date	Name of study or source of participants	Location	Study design	Baseline population	Year of baseline survey	Baseline age range (years)	% male	Follow up (years)	Outcome (s)	Total participants	Covariates adjusted for	Study quality
Maser, 2015	Diabetes and Metabolic Research Center	USA	Cross-sectional	Type 2 diabetes	NR	≥ 18	42.0	NA	CAC	50	NA	5
Yeap, 2015	HIMS	Australia	Prospective cohort	Community-based men	2001-2004	70-89	100.0	NA	CHD, stroke	3,384	Education, smoking, BMI, WHR, hypertension, dyslipidemia, diabetes, creatinine, vitamin D, prevalent CVD, cancer	7
Zhang, 2015	Hospital	China	Cross-sectional	Non-dialysis with CKD	NR	50-75	62.9	NA	Carotid atherosclerosis	240	Age, sex, BMI, smoking history, MABP, eGFR, therapeutic medication use, and FBG, TC, TG, LDL-C, HDL-C, and hs-CRP levels	6
Kim, 2016	Hospital	South Korea	Cross-sectional	Patients who underwent CABG	2012--2013	60.7*	100.0	NA	Coronary artery stenosis	122	NA	5
Deng, 2018	Hospital	China	Cross-sectional	Patients who underwent screening for carotid atherosclerosis	2014-2016	40-60	100.0	NA	Carotid atherosclerosis	335	NA	3
Ling, 2018	Hospital	China	Cross-sectional	Post-menopausal women with CAD and controls	2015-2016	66.0*	0.0	NA	CHD, coronary atherosclerosis	227	Age, BMI, smoking, hypertension, diabetes, LDL-C, HDL-C, TG, eGFR, statin use and high-sensitivity CRP	5
Zhang, 2018	Hospital	China	Retrospective cohort	Coronary angiography patients	2008-2009	65.5*	66.4	4.4	CVD	247	Age, BMI, WC, SBP, DBP, FPG, 2hPG, HOMA-IR, TG, HDL-C, LDL-C, CRP, current smoker	6

AAC, abdominal aortic calcification; ACS, aortic calcification score; BMD, bone mineral density; BMI, body mass index; CABG, coronary artery bypass graft; CAC, coronary artery calcification; CAD, coronary artery disease; CHD, coronary heart disease; CHF, congestive heart failure; CKD, chronic kidney disease; CIMT, carotid artery intima-media thickness; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate, FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; HIMS, Health In Men Study; HOMA-B, homeostasis model assessment of beta cell function; HOMA-IR, homeostasis model assessment of insulin resistance; HPC, Health Promotion Center; LASA, Longitudinal Aging Study Amsterdam; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; NA, not applicable; NR, not reported; SBMS, Severance Bone Metabolism study; SBP, systolic blood pressure; SOF, Study of Osteoporotic Fractures; SOS, Shangai Obesity Study; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WHR, waist-to-hip ratio

SUPPLEMENTARY MATERIAL

Appendix 1	PRISMA checklist
Appendix 2	MOOSE checklist
Appendix 3	MEDLINE literature search strategy
Appendix 4	Modified Newcastle Ottawa Quality Scale for cross-sectional studies
Appendix 5	Reference list of included studies

Appendix 1. PRISMA checklist

Section/topic	Item No	Checklist item	Reported on page No
Title			
Title	1	Identify the report as a systematic review, meta-analysis, or both	1
Abstract			
Structured summary	2	Provide a structured summary including, as applicable, background, objectives, data sources, study eligibility criteria, participants, interventions, study appraisal and synthesis methods, results, limitations, conclusions and implications of key findings, systematic review registration number	2
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS)	4
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (such as web address), and, if available, provide registration information including registration number	4
Eligibility criteria	6	Specify study characteristics (such as PICOS, length of follow-up) and report characteristics (such as years considered, language, publication status) used as criteria for eligibility, giving rationale	4
Information sources	7	Describe all information sources (such as databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated	Appendix 3
Study selection	9	State the process for selecting studies (that is, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis)	4-5
Data collection process	10	Describe method of data extraction from reports (such as piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators	5
Data items	11	List and define all variables for which data were sought (such as PICOS, funding sources) and any assumptions and simplifications made	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis	5
Summary measures	13	State the principal summary measures (such as risk ratio, difference in means).	5-6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (such as I^2 statistic) for each meta-analysis	5-6
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (such as publication bias, selective reporting within studies)	6
Additional analyses	16	Describe methods of additional analyses (such as sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified	6
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram	6 and Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (such as study size, PICOS, follow-up period) and provide the citations	6-7, Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome-level assessment (see item 12).	7, Table 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present for each study (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a forest plot	7-10
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency	7-10, Figures 2-4
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see item 15)	7, Table 1
Additional analysis	23	Give results of additional analyses, if done (such as sensitivity or subgroup analyses, meta-regression) (see item 16)	NA
Discussion			
Summary of evidence	24	Summarise the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (such as health care providers, users, and policy makers)	10-11
Limitations	25	Discuss limitations at study and outcome level (such as risk of bias), and at review level (such as incomplete retrieval of identified research, reporting bias)	12-13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research	13
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (such as supply of data) and role of funders for the systematic review	None

Appendix 2. MOOSE checklist

Association of circulating osteocalcin with cardiovascular disease and intermediate cardiovascular phenotypes: systematic review and meta-analysis

Criteria		Brief description of how the criteria were handled in the review
Reporting of background		
√	Problem definition	The data on the associations of circulating osteocalcin (OC) with cardiovascular disease (CVD) are sparse and conflicting. In this context, we have carried out a comprehensive systematic meta-analysis to quantify the associations of circulating OC (total, undercarboxylated, and carboxylated with cardiovascular outcomes (clinical CVD endpoints and intermediate cardiovascular phenotypes).
√	Hypothesis statement	Circulating levels of OC are associated with cardiovascular outcomes
√	Description of study outcomes	CVD-related outcomes [composite CVD, coronary heart disease (CHD), stroke, congestive heart failure (CHF), or all-cause mortality]; and (ii) intermediate cardiovascular traits [carotid atherosclerosis, aortic atherosclerosis, coronary artery calcification (CAC), carotid intima-media thickness (CIMT), abdominal aortic calcification (AAC)].
√	Type of exposure	Blood circulating levels of OC (total OC, undercarboxylated OC, and carboxylated OC
√	Type of study designs used	Observational cohort, case-control, or cross-sectional population-based studies
√	Study population	Healthy participants, pre- and post-menopausal women, as well as participants with pre-existing conditions such as metabolic syndrome, type 2 diabetes, and participants at high cardiovascular risk
Reporting of search strategy should include		
√	Qualifications of searchers	Samuel Seidu, MD; Setor Kunutsor, PhD
√	Search strategy, including time period included in the synthesis and keywords	Time period: from inception of MEDLINE and EMBASE to 22 March 2019. **Search strategy:** The detailed search strategy can be found in Appendix 3.
√	Databases and registries searched	MEDLINE and EMBASE
√	Search software used, name and version, including special features	OvidSP was used to search EMBASE EndNote used to manage references
√	Use of hand searching	We searched bibliographies of retrieved papers
√	List of citations located and those excluded, including justifications	Details of the literature search process are outlined in the flow chart. The citation list for excluded studies is available upon request.
√	Method of addressing articles published in languages other than English	We placed no restrictions on language
√	Method of handling abstracts and unpublished studies	Not applicable
√	Description of any contact with authors	We contacted authors who had conducted univariate or multivariate analysis with osteocalcin as an exposure and cardiovascular outcomes but had not reported relevant estimates.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Detailed inclusion and exclusion criteria are described in the Methods section.
√	Rationale for the selection and	Data extracted from each of the studies were relevant to the

	coding of data	population characteristics, study design, exposure, outcome, and possible effect modifiers of the association.
√	Assessment of confounding	We assessed confounding by ranking individual studies on the basis of different adjustment levels.
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Study quality was assessed based on the nine-star Newcastle–Ottawa Scale using pre-defined criteria namely: population representativeness, comparability (adjustment of confounders), ascertainment of outcome.
√	Assessment of heterogeneity	Limited data precluded assessment of heterogeneity
√	Description of statistical methods in sufficient detail to be replicated	Description of methods of meta-analyses and assessment of publication bias are detailed in the methods. We performed random effects meta-analysis with Stata 14.
√	Provision of appropriate tables and graphics	See Figures 2-4; Table 1
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	Figures 2-4
√	Table giving descriptive information for each study included	Table 1
√	Results of sensitivity testing	NA
√	Indication of statistical uncertainty of findings	95% confidence intervals were presented with all summary estimates
Reporting of discussion should include		
√	Quantitative assessment of bias	The systematic review is limited in scope, as it involves published data. Individual participant data is needed. Limitations have been discussed.
√	Justification for exclusion	All studies were excluded based on the pre-defined inclusion criteria in methods section.
√	Assessment of quality of included studies	Brief discussion included in 'Methods' section
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	We discussed that potential unmeasured confounders may have caused residual confounding. Additionally, our findings could have been over-estimated somewhat due to preferential publication of extreme findings. The variations in the strengths of association may also be due to true population differences, or to differences in quality of studies.
√	Generalization of the conclusions	Discussed in the context of the results.
√	Guidelines for future research	We recommend large-scale prospective data
√	Disclosure of funding source	No separate funding was necessary for the undertaking of this systematic review.

Appendix 3. MEDLINE literature search strategy

1 exp OSTEOCALCIN/bl [Blood] (3992)
2 exp Cardiovascular Diseases/ (2257611)
3 exp Coronary Disease/ (209108)
4 exp Coronary Artery Disease/ (56488)
5 exp STROKE/ 120515 ()
6 exp ATHEROSCLEROSIS/ (38589)
7 exp Carotid Intima-Media Thickness/ (4196)
8 exp Vascular Calcification/ (3505)
9 aortic calcification.mp. (1019)
10 exp MORTALITY/ (356269)
11 exp Heart Failure/ (112241)
12 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 (2536035)
13 1 and 12 (155)
14 limit 13 to (humans and "all adult (19 plus years)") (126)

Each part was specifically translated for searching alternative databases.

Appendix 4. Modified Newcastle Ottawa Quality Scale for cross-sectional studies

The methodological quality score is based on New-Castle Ottawa Quality Scale and is adapted for this review. Maximum of one star can be awarded for each item in Selection and Outcome categories. A maximum of two stars can be given for Comparability items.

Cut-off scores
Low methodological quality 0-3 stars
Moderate methodological quality 4-6 stars
High methodological quality 7-8 stars (>75%)

Category 1: Selection

1. Representativeness of the sample
(a) Truly representative if the sample is randomly derived from the general population with sample size of >100 subjects *
(b) Somewhat representative sample from the population with sample size of >100*
(c) Selected group of users (e.g., nurses, volunteers)
(d) No description of the derivation of the cases.

2. Non-respondents
(a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory*
(b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory
(c) No description of the response rate or the characteristics of the responders and the non-responders

3. Adequate definition of exposure
(a) Yes, according to a clear and widely used definition *
(b) Yes, from record linkage or based on self-reports
(c) No description.

4. Ascertainment of exposure
(a) Secure record*
(b) Written self-report
(c) No description

Category 2: Comparability

5. Comparability on the basis of the design/analysis
(a) Study controls for age, sex, or BMI*
(b) Study controls for any additional factor: Smoking status, education, alcohol intake, physical activity, lipids, or blood pressure)*

Category 3: Outcome

6. The study used a precise definition of outcome and valid and reliable method (individually for each relevant outcome)

7. Assessment of outcome
(a) Independent blind assessment (reference to medical records)*
(b) Record linkage (coded by ICD on database records)*
(c) Self-report.
(d) No description.

8. Statistical test
(a) The statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is present, including confidence intervals and the probability level (p-value)*
(b) The statistical test is not appropriate, not described or incomplete.

Appendix 5. Reference list of included studies

1. Jie KS, Bots ML, Vermeer C, Witteman JC, Grobbee DE. Vitamin K intake and osteocalcin levels in women with and without aortic atherosclerosis: a population-based study. *Atherosclerosis.* 1995;116(1):117-123.
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